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The human intestinal B-cell response

Jo Spencer¹ and Ludvig M. Sollid²

¹Peter Gorer Department of Immunobiology, King's College London, London, UK

²Centre for Immune Regulation and Department of Immunology, University of Oslo and Oslo University Hospital-Rikshospitalet, Oslo, Norway.

The authors contributed equally to the manuscript and both authors are corresponding authors.

Jo Spencer
Peter Gorer Department of Immunobiology,
King's College London,
Guy's Hospital Campus,
London,
SE1 9RT.

Email: jo.spencer@kcl.ac.uk
Telephone: + (44) 20 7188 1614
FAX: + (44) 20 7188 3385

Ludvig M Sollid
Centre for Immune Regulation and Department of Immunology,
University of Oslo and Oslo University Hospital-Rikshospitalet,
PO Box 4950 Nydalen,
Oslo 0424,
Norway.

Email: l.m.sollid@medisin.uio.no
Telephone: + (47) 23 07 38 11
FAX: *Fax*: + (47) 23 07 35 10

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Abstract

The intestinal immune system is chronically challenged by a huge plethora of antigens derived from the lumen. B-cell responses in organised gut-associated lymphoid tissues (GALT) and regional lymph nodes that are driven chronically by gut antigens generate the largest population of antibody producing cells in the body; the gut lamina propria plasma cells. Although animal studies have provided insights into mechanisms that underpin this dynamic process, some very fundamental differences in this system appear to exist between species. Importantly, this prevents extrapolation from mice to humans to inform translational research questions. Therefore, in this review we will describe the structures and mechanisms involved in the propagation, dissemination and regulation of this immense plasma cell population in man. Uniquely, we will seek our evidence exclusively from studies of human cells and tissues.

Introduction

Immune protection of the human intestinal mucosa depends on the secretion of IgA by plasma cells that occupy the diffuse subepithelial connective tissue of the intestinal lamina propria. Locally secreted IgA bound to polymeric immunoglobulin receptor (pIgR) is actively transported into the gut lumen where it regulates bacterial populations.^{1, 2} The body commits considerable resources to the production of intestinal IgA. The number of IgA producing plasma cells, at approximately 6×10^{10} , exceeds the total number of plasma cells in the rest of the body combined, including bone marrow, lymph nodes and spleen.^{1, 3} Intestinal plasma cells secrete between 3 and 5g of IgA per day that is actively transported into the gut lumen to provide continuous protection of the mucosal barrier. Human intestinal IgA is mostly dimeric and comprises both the IgA1 and IgA2 subclass. The ratio between plasma cells secreting the two subclasses of IgA is not the same through the gastrointestinal tract. Whereas most plasma

cells in the small intestine secrete IgA1, the proportion of IgA2 increases from the duodenum through to the terminal ileum and the subclasses are present in approximately equal numbers in the colon (Figure 1). IgA2 has a shorter hinge than IgA1 and as a consequence is less susceptible to bacterial proteases and would therefore have a functional advantage in the lumen of the colon that is rich in bacterial species. In contrast, whilst IgA is the second most common immunoglobulin isotype in blood after IgG, most IgA in serum is monomeric IgA1 (ref¹).

The precursors of lamina propria IgA plasma cells are generated in microanatomically discrete zones of organised lymphoid inductive sites such as the gut-associated lymphoid tissue (GALT) and gut regional lymph nodes (Figure 1). First we will consider the structure and function of human GALT and then the derivation and unique properties of intestinal plasma cells themselves.

B cells in gut-associated lymphoid tissue

Most of the intestine is structured to prevent the entry of potentially pathogenic material into the body, for example by epithelial tight junctions to maintain the integrity of the monolayer even through rapid cell turnover and the production of anti-microbial peptides such as defensins.^{4, 5} In contrast, GALT is associated with an epithelial structure that facilitates antigen entry (Figure 1). Antigens may be sampled by epithelial cells termed microfold (M) cells that comprise part of the specialised follicle associated epithelium (FAE).⁶ GALT covered by an FAE may be located anywhere through the GI tract but tends to be concentrated in the terminal ileum and the appendix.⁷ In these locations GALT comprises lymphoid follicles that tend to be located on the mucosal aspect of the muscularis mucosae with a broad and relatively exposed FAE⁸. In colon most of the lymphoid tissue tends to be concentrated on the

serosal side of the muscularis layer, protruding through a hole in the muscularis and forming a narrow FAE interface with the lumen.⁹

The FAE interacts intimately with the lymphoid tissue below and is infiltrated by lymphocytes, including B cells, from approximately 20 weeks of gestation onwards.^{10, 11} The amount of lymphoid tissue present in the gut increases through the last 20 weeks of gestation, with acquisition accelerating after birth and continuing to increase through to 12 years of age, after which the number of lymphoid follicles declines so that their frequency reaches levels present at birth in the 8th decade.¹²

Gut-associated lymphoid tissue contains diverse B-cell subsets. The central feature of most GALT in the small intestine and appendix are the prominent germinal centers (GC) containing B cells that have been activated. B cells in GC are surrounded by other cells such as follicle dendritic cells, T follicular helper cells and tingible body macrophages that support GC B-cell clonal expansion and selection (Figure 2). The ubiquitous presence of a GC in small intestinal GALT is likely a reflection of the chronic stimulation by antigens derived from the lumen via the FAE and the high antigen load. Pockets of cells that have features of GC B cells have been described in FAE¹³ suggesting unique features of B-cell responses with FAE location. Colonic GALT less consistently contains a GC though the likelihood of the presence of GC in colonic GALT is increased by the presence of a colonic tumor.⁹

The GC is surrounded by a mantle zone of CD27-IgM+IgD+ mature naïve B cells that is generally sparse in GALT compared to the tonsil or a lymph node. The mantle has its broadest aspect facing the direction of antigen entry; either the FAE of GALT or the subcapsular sinus of lymph node. The mantle zone of GALT is surrounded by a marginal zone of B cells that lack the strong expression of IgD of mantle zone cells, and that extends to and infiltrates the

adjacent FAE.¹¹ The GALT marginal zone B cells are considered to be the benign analogue of the malignant B cells in marginal zone B-cell lymphoma of MALT (MALT lymphoma).¹⁴ Normal FAE retains a tight structure despite mild disruption by infiltrating lymphocytes. In contrast, the lymphoepithelium formed by MALT lymphoma is a destructive lesion that is pathognomonic of the tumor.¹⁴

B cells outside gut-associated lymphoid tissue in the lamina propria

Whereas CD20+ B cells are numerous in GALT structures, they are scarce in the lamina propria (Figure 3). A few scattered B cells (CD20+, CD27+, MHCII+), mainly IgA expressing, can be observed in sections of jejunal tissue. This is in contrast to what is observed by flow cytometry analysis of single cell suspension made from resection specimens. In this case, numerous B cells including sIgD+ naïve B cells, can be observed. Thus contamination of cells from GALT structures is an important confounder in analysis of single cell suspension of mucosal tissue samples. B lineage effector cells secreting antibodies, i.e. plasma cells and plasmablasts, have an almost exclusive distribution in the gut in the lamina propria.¹⁵ IgA is by far the dominant isotype produced by these cells. In duodenum/jejunum, 79% of plasma cells express IgA, 18% express IgM and 3% express IgG, whereas the corresponding numbers in colon are 90%, 6% and 4%.¹ In the small intestine most of the plasma cells are localized in a zone stretching from the luminal 100 µm of the crypt layer to the basal 100 µm of the villi, a zone intermingled with a few T cells.¹ Initial observations by Farstad et al reported that some IgA immunocytes express surface immunoglobulin and it was suggested that these antibody-secreting cells represent recently arrived plasmablasts.¹⁵ Benkert et al also termed these cells plasmablasts as the authors found, similar to what had been observed by Di Niro et al, that virtually all gut IgA and IgM ASC express surface immunoglobulin.^{16, 17} Notwithstanding, these cells generally lack intracellular expression of proliferation-associated protein Ki-67 and express CD138 indicating that they are plasma cells rather than plasmablasts.¹⁷ Further in

support of the notion that these are not plasmablasts captured in narrow window of differentiation, it was observed that surface immunoglobulin is maintained on antibody-secreting cells in culture of gut biopsies even after 4 weeks.¹⁸ The expression of surface IgA and IgM is not unique to gut antibody secreting cells as this feature is also observed in plasma cells of the bone marrow.¹⁹ In fact, the surface immunoglobulin of IgA and IgM plasma cells appears to be a functional B-cell receptor that is associated with Ig α /Ig β . Consistent with this, cross-linking of IgA and IgM on the surface of plasma cells gives Ca²⁺ mobilization and extracellular signal-regulated kinase 1/2 and AKT phosphorylation. B-cell receptor cross-linking furthermore leads to increased survival of IgA plasma cells as measured by intact immunoglobulin secretion after 4 days.¹⁹ In contrast to IgG plasma cells that are located at sites (bone marrow and spleen) mostly devoid of antigen, IgA and IgM plasma cells are primarily located at mucosal sites where they can be in contact with antigen. Thus, sensing of antigen by IgA and IgM plasma cells using their surface antigen receptor could help guiding production of secretory immunoglobulin for which there is a need both by stimulating antibody production and by securing cell survival. Plasma cell survival could either happen directly as indicated by Pinto et al or indirectly via T cells due to antigen presentation (Figure 4). As to the latter, plasma cells have barely detectable MHC class II expression. Notwithstanding, if the few MHC class II molecules are loaded with peptides from antigen extremely efficiently taken up by the cell via the B-cell receptor this could be sufficient to receive T-cell help. The two mechanisms are not mutually exclusive, and further work is needed to determine whether these mechanisms operate in the gut.

High dimension analysis of B cells in the intestine by single-cell mass cytometry demonstrated that intestinal antibody secreting cells generally have low expression of chemokine receptors C-C motif receptor 6 (CCR6) and CCR7 and high expression of CCR10 and CXCR4, i.e. they carry the phenotype CCR6^{lo}/-CCR7^{lo}/-CCR10⁺ CXCR4⁺ (ref ²⁰). The expression of the adhesion

molecule $\alpha 4\beta 7$ integrin in intestinal antibody secreting cells is lower than of antibody secreting cells in blood. Further, intestinal and circulating antibody secreting cells express lower levels of surface IgA and CD40 than switched memory B cells, and interestingly, in contrast to circulating antibody secreting cells that are HLA-DR⁺/CD95⁺, intestinal plasma cells are generally HLA-DR⁻CD95⁻. This suggests that the cells would be non-responsive to apoptosis via CD95 ligation.²⁰

Function of human gut-associated lymphoid tissue

Precursors of human IgA plasma cells originate from the GC of GALT. Examples of clones of B lineage cells (where clones are identified by the homology of junctional sequences of their rearranged IGHV genes) that are present in both the GC of GALT and also in the lamina propria plasma cell population have been identified.²¹ Probably the best evidence for involvement of the GC in the history of plasma cells in health is the very high frequency of somatic mutations in the rearranged IGHV genes of virtually all human lamina propria plasma cells in health.²² The GC is the only place known where somatic mutations are introduced with high frequency into IGV genes. The AID enzyme that catalyses the mutation of IGV gene segments to generate variable region variants is expressed in the nuclei of centroblasts in GALT GCs and also in the cytoplasm of relatively sparse large interfollicular B cells, the significance of which is not known.^{23, 24} The reason that a high frequency of somatic mutation is acquired and tolerated such that on average approximately 8% of the variable region sequence is changed, despite selective pressure to maintain structural viability, is not known. It may be a consequence of drive from the high antigenic load, the chronicity of the challenge, or lower selective pressure so that a greater range of variants is tolerated.

Despite the apparent ubiquitous involvement of GCs in GALT plasma cell development, it is likely that both T-cell independent type 2 antigens (TI2) that are not conventionally

associated with initiation of GC responses and T-cell dependent antigens can both drive B-cell activation in GALT. The gut is rich in bacterial polysaccharide antigens with repeating subunit structures that could potentially initiate TI2 responses.²⁵

One of the most distinctive and often unappreciated features of human GALT marginal zone B cells is their expression of Fc receptor-like 4 (FCRL4, previously IRTA1 or FCRH4)²⁶ that was originally identified by the translocation of the encoding gene to the Ig locus in MALT lymphoma.²⁷ This receptor, that does not occur in mice, has ITIM motifs and dampens polyclonal immune responses, for example to B cell receptor signalling or *Staphylococcus aureus* Cowan strain. This suggests that the B cells on the mucosal front line in humans have distinctive raised thresholds of responsiveness.^{28, 29} It has also been demonstrated that FCRL4 may detach B cell responses from the activation signals normally driven through BCR ligation in favour of responses through innate receptors.³⁰ In health FCRL4 expressing B cells are rare in blood and lymphoid tissues distant from epithelia. However they accumulate systemically in HIV and in individuals from malaria endemic zones.^{31, 32} When systemic B cells express FCRL4, this B cell subset is also refractory to stimulation and has been described as 'exhausted'. The FCRL4 expressing populations in blood in HIV and malaria tend to be CD27-^{31, 32} Interestingly, FCRL4+ cells with proinflammatory properties are present in rheumatoid synovium suggesting functional similarity between B cells in the gut and the rheumatoid joint. This FCRL4+ population is predominantly IgD- and includes CD27+ and CD27- memory subsets.³³

The induction of GCs conventionally requires cognate interaction between B cells and T cells involving the ligation of CD40 on B cells by CD40L on peptide primed T cells. The failure of this in X-linked hyper IgM type 1 due to mutations in CD40L results in deficiency in GC formation and dearth of class switched B cells systemically. Class switch to IgA can however

be retained despite CD40L deficiency.³⁴ Studies of blood from patients with CD40L deficiency have identified that CD27-IgA⁺ B cells derived in the absence of cognate T cell help that are present in similar frequency to healthy controls, consistent with a T-cell independent pathway for generation of IgA expressing memory cells.³⁴ CD27⁻ class switched memory B cells are also increased in relative frequency in patients depleted of T cells by HIV.³⁵ Circulating CD27⁻, IgA⁺ B cells in health have a bias towards lambda light chain usage, a relatively low frequency of somatic mutations and relatively more polyreactive immunoglobulins with a greater tendency to bind bacterial species than CD27⁺IgA⁺ cells. They also have a gene expression profile suggesting ability to traffic to intestinal sites.^{34, 36} Several lines of evidence described above tie a phenotype of CD27⁻ memory B cells with circumstances of B cell priming in the absence of cognate T-cell help. Whilst phenotypic profiles particularly involving CD27 have been proposed to discriminate between B cells differentiating along T-cell dependent and independent pathways, others have observed that memory B cell clones commonly have both CD27⁺ and CD27⁻ members.³⁷ This will be an interesting area to observe in the future.

IgA class switch recombination

The presence of IgA in patients with hyper IgM syndromes suggests that class switch recombination (CSR) to IgA can be independent of cognate B-cell T-cell interactions,^{34, 38} though such patients often have small residual GC structures that could potentially support classically GC dependent processes.³⁹

Class switch to IgA requires cell proliferation, induction of the enzymes that mediate the recombination process such as AID and also switch factors that initiate the production of transcripts from the noncoding switch regions upstream of the Igα1 and Igα2 constant region segments (**Figure 5**). Double strand breaks in the switch regions initiated by the deamination

of cytidine in the repeating motifs that guide the activity of AID facilitate the exchange of constant region segments in dividing cells. Crosslinking CD40 is a potent stimulator of cell division that can also activate NFκB resulting in upregulation of AID to mediate CSR to all isotypes in the presence of appropriate switch factors.⁴⁰ It has been demonstrated that ligation of TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) by APRIL (a proliferation inducing ligand) can also activate NFκB and induce AID by recruitment of MyD88 to a cytoplasmic domain of TACI.^{41, 42} It is also possible that innate ligands such as CpG may drive cell division and CSR in memory cells in the absence of cognate interaction with T cells.²⁵

The first switch factor observed to induce IgA CSR through the induction of transcription of Igα switch regions was TGFβ.⁴³ Other cytokines including IL10 and IL4 and retinoic acid have also been implicated in supporting CSR or the maturation of switched B cells.^{44, 45} Most recently IgA inducing protein (IGIP) produced by dendritic cells, that have previously been implicated in the induction of IgA CSR,^{38, 46} has been demonstrated to induce IgA switching.⁴⁷ The production of IGIP by dendritic cells is regulated by vasoactive peptide (VIP).⁴⁷

It is currently unclear how preferential switching to either IgA1 or IgA2 along the intestine is initiated. Recombination to each is usually direct. Despite its downstream location to IgA1, switch to IgA2 is unlikely to be a second step following an intermediate switch to IgA1 (ref⁴⁸). Interestingly higher frequencies of IgA2 secreting cells are observed in locations of high bacterial load, suggesting that preferential switch to IgA2 may be a feature of the inducing antigen.^{1, 49}

During the first month of life IgA secreting plasma cells are virtually absent from the intestinal lamina propria. Their subsequent presence increases over the first two years of life

simultaneous with the expression of APRIL and expression of its receptors TACI and BCMA (B-cell maturation antigen) in GALT. The association between IgA plasma cell appearance and the presence of APRIL and its receptors suggests that this axis is important in plasma cell development in early life.²⁴ Epithelial APRIL expression may be modulated by the ligation of TLR5 by flagellin, suggesting that the microbiome could also promote IgA CSR indirectly.⁴¹

Function of secretory antibodies

The plasma cells of the gut express the J-chain regardless of the isotype produced.⁵⁰ The J-chain is instrumental to make dimeric IgA and pentameric IgM, and it does so by forming covalent disulphide bridges with the C-terminal parts of the α and μ heavy chains.⁵¹⁻⁵³

Polymeric IgA and IgM by means of their J-chain association with pIgR expressed on the basolateral side of enterocytes^{54, 55} This receptor mediates immunoglobulin transport across the epithelium. Secretory antibodies are released by cleavage of the pIgR at the luminal membrane. In the lumen the released antibodies can bind antigens including gut microbes,² and thereby mediate immune exclusion and antigen clearance. ⁵⁶ Of note, the pIgR can also transport preformed immune complexes⁵⁷ and intracellular dimeric IgA can bind and neutralize lipopolysaccharide thereby playing an anti-inflammatory role.⁵⁸

The pIgR is not the only receptor for IgA on enterocytes. The transferrin receptor (CD71) does also bind IgA1.⁵⁹ The transferrin receptor is primarily expressed on the apical side of enterocytes, and it can bind and mediate retrotranscytosis of secretory IgA.⁶⁰ This could be a way to facilitate uptake of antigens across the epithelial barrier to which there is an already established humoral response like to gluten in celiac disease.⁶⁰

From GALT to the lamina propria via the blood.

Plasmablasts derived from B cells activated in GALT can circulate via the blood and home back to the gut. Homing is mediated by a combination of lymphocyte homing receptors for endothelial ligands such as $\alpha 4\beta 7$ integrin receptor for mucosal endothelial MAdCAM and chemokine receptors for chemokines secreted by intestinal epithelial cells such as CCR9 and CCR10 that facilitate migration towards CCL25 and CCL28 respectively.⁶¹ CCL25 and CCL28 are produced dominantly by the small intestinal and colonic epithelium respectively, thus providing a mechanism to bias homing of effector cells to either small intestine or colon (Figure 1). $\alpha 4\beta 7$ and CCR9 are inducible by retinoic acid generated from vitamin A substrate by the RALDH enzyme of GALT dendritic cells.⁶² Vitamin D3 metabolite 1,25-Dihydroxyvitamin D3 may induce CCR10 and thus guide migration of plasma cell precursors preferentially to colon.⁶³ Mucosal immunisation with cholera toxin, either per oral or rectally generated circulating antibody forming cells expressing the $\alpha 4\beta 7$ integrin predominantly.⁶⁴ Similarly, antibody-producing cells generated in response to oral mucosal immunisation with salmonella vaccine induced cells in blood that migrated towards chemokines CCL25 and CCL28 (ref⁶⁵).

A molecule located in basement membrane surrounding the high endothelial venules (HEV) of GALT but not HEV of peripheral lymph nodes or tonsils has been identified. Since lymphocytes traffic through HEV is it possible that this molecule could be involved in selective recruitment of cells into intestinal tissues.^{66, 67}

Relationship of B-cell responses in the gut to systemic humoral responses

In steady state in non-immunized subjects, 80% of antibody secreting cells in peripheral blood express IgA as well as the chemokine receptor CCR10 and $\alpha 4\beta 7$ integrin.⁶⁸ This suggests a dominant presence in blood of antibody secreting cells that originate from mucosal immune responses and also these cells should be able to return to mucosal sites, though they

were also identified in bone marrow. Interestingly, upon parenteral tetanus/diphtheria vaccination, vaccine specific intracellular IgG cells was observed in blood 8 days after vaccination, but no similar surge of IgA plasmablasts could be observed. This may indicate that there is independent regulation of systemic and mucosal humoral immune responses.⁶⁸ Similarly, it was observed that following anti-CD20 (rituximab) therapy, IgA plasmablasts were still maintained in blood and plasma cells could be identified in the gut lamina propria suggesting that the B-cell precursors of these cells were not affected by the anti-CD20 therapy.⁶⁹ Of note, the maintenance of plasma cells in the gut lamina propria could also be explained by some gut plasma cells having long life spans (see below).

Evidence for disconnection of mucosal and systemic humoral responses, at least to some degree, has also been obtained by staining of B cells and plasma cells with fluorescent virus-like particles of rotavirus. In a study of peripheral blood of healthy subjects 0.6% of the B cells stained with rotavirus VP6 particles, but only about 10% of these cells carried the $\alpha 4\beta 7$ gut homing receptor.⁷⁰ The rotavirus specific B cells had a bias in usage of VH gene segments when compared with random memory B cells or B cells of gut homing phenotype, particularly VH1-46 but also VH4-31 and VH4-39. Among all gut homing B cells there was bias for VH4 gene family usage, particularly VH4-39. Interestingly, rotavirus specific lamina propria plasma cells isolated with the same type of virus-like particles have a bias for VH4 usage particularly VH4-39 (refs ^{17, 20}). These results, though not comprehensive, may suggest that the VH usage of B cells that later home to and operate in the gut as plasma cells is influenced by antigen, but possibly also by the mucosal priming.

It has been suggested that the marginal zone B-cell subset that is normally resident in spleen and that is responsible for systemic immune responses to bacterial polysaccharide antigens in humans may develop in GALT.⁷¹ Human splenic marginal zone B cells (unlike those in mice)

have mutated IGHV genes suggestive of previous antigen encounter and their origin remains enigmatic.⁷² Consistent with a role for the gut in development of the systemic B-cell repertoire, Vossenkamper et al observed that immature transitional type 2 B cells are present in GALT and that they are activated in this microenvironment.⁷³ This study also described that B cells in most cases of the systemic autoimmune disease systemic lupus erythematosus that have been reported to be defective in mechanisms that regulate peripheral B cell tolerance⁷⁴ are compromised in their ability to enter the gut through their reduced expression of $\alpha 4\beta 7$ integrin. The significance of this and any possible link to the development of marginal zone B cells remains to be determined.

Antibody repertoire and reactivity of gut plasma cells

The immunoglobulin repertoire of the human adult gut plasma cells is highly diverse with a high degree of somatic mutations.^{75, 76} The number of somatic mutations increases with age suggesting that cumulated antigen exposure contributes to accumulation of mutations over time.^{75, 77} Interestingly, the degree of mutations is as high in plasma cells of the duodenum as of the colon despite the fact that bacterial colonization is scarce in the proximal gut.⁷⁵ Dietary antigens are likely the major drivers for upper small bowel humoral responses. Thus to explain this observation, either dietary antigens give rise to highly mutated responses or alternatively, plasma cells originating from a B-cell clone could have wide distribution over the gut. Gluten-specific plasma cells in celiac disease have limited mutations of their antibody genes,⁷⁸ and with the reservation that celiac disease response might not be representative of antibody responses to dietary antigens in general, this lends some credence to the latter explanation. In fact this notion of widespread dissemination of plasma cells has received experimental support from many, but not all studies. Early work employing complementarity determining region (CDR)-3 spectratyping and sequence analysis suggested clonal sharing between plasma cells in the small bowel and colon⁷⁵ as well as between multiple distinct sites

in the colon.⁷⁹ Recent work by use of high throughput immunoglobulin VH gene sequencing demonstrated substantial clonal sharing between plasma cells of distinct duodenal biopsies of celiac disease patients, most notable for transglutaminase 2 specific cells but also for non-transglutaminase 2 specific cells.⁸⁰ Contrary to these observations, Yuvaraj et al by sequence analysis of IgA VH genes of ileal biopsies observed many clonally related sequences in one biopsy, but not between different biopsies.⁸¹ The authors interpreted this finding to support the notion of local expansion of IgA precursor cells in the lamina propria, and suggested that there could be a first wave of memory B cells originating in the GCs that seed into the lamina propria and that these cells then expand locally. In a related but distinct model, He et al suggested that colon lamina propria B cells by the influence of enterocyte produced APRIL in a T-cell independent manner can switch from IgM to IgA2 and then differentiate locally to become plasma cells.⁴¹ In vitro experiments gave support for this model. However, phenotypic analysis of lamina propria B cells in situ provided little support for neither of these models. Boursier et al observed no signs of local proliferation of CD20+ or CD19+ cells in the lamina propria and found no evidence of AID mRNA expression in lamina propria outside the organized gut associated lymphoid tissue;⁸² the latter finding being consistent with more recent observations.⁸⁰ Thus it appears that the majority of gut plasma cells are generated by GC reactions, giving CSR and somatic mutations, and afterwards the plasma cells are seeded into the lamina propria in wide areas of the gut.

Another useful source of information for understanding gut humoral immunity is sequencing in large scale of immunoglobulin heavy and light chain genes of single plasma cells and testing of the reactivity of reconstituted recombinant monoclonal antibodies. Benkert et al sequenced IgA and IgG plasma cells from the terminal ileum of healthy subjects.¹⁶ They found that all antibodies, both IgA and IgG, had high numbers of somatic mutations. Again, evidence of clonal expansions was observed; about 4% of all IgA and 12% of all IgG antibodies were

assigned to expanded clones, but clonally related B cells were not shared between the IgA⁺ and IgG⁺ plasma cell compartments. Despite some low level of cross-reactivity with several bacterial species, and some degree of polyreactivity to self-antigens, both IgA and IgG plasma cells in general expressed antibodies that were highly specific. Specific reactivity of recombinant antibodies were also observed by isolation of rotavirus specific plasma cells from healthy subjects¹⁷ as well as by transglutaminase 2 and gluten specific plasma cells of celiac disease patients.^{78, 80} Taken together these results strongly indicate that gut plasma cells are generated in response to specific antigens, and they do not give support to a concept of the existence of scarcely mutated antibodies that have a broad reactivity, for instance to commensal bacteria in general. It has been suggested that polyreactivity so often cited as a property of IgA could be a consequence of nonspecific binding through carbohydrate groups.⁸³ This interesting field remains open to further investigation.

Immunoglobulin heavy chains are expressed with either kappa or lambda light chains. Whereas the ratio of IgK:IgL for serum IgG is approximately 1.7:1, the figure for IgA is 1.2:1 (ref⁸⁴). As discussed above, a bias towards expression of IgL is also seen in B cells expressing IgA, in particular memory cells lacking CD27 (ref³⁴). It has been suggested that a movement of B cells from expression of kappa light chain to lambda light chain could occur by receptor revision where B cells outside the bone marrow re-express RAG proteins that mediate secondary light chain gene rearrangements. Perhaps the best evidence for this is the bias towards the most 3' J segments in lambda excision circle by-products of the rearrangement process isolated from IgA plasma cells.⁸⁵ Light chain drifting could be a mechanism that diversifies the antigen-binding repertoire of intestinal immunoglobulins alongside somatic hypermutation, and could be a particular feature of CD27⁻ memory cells that may be independent of cognate interactions through CD40 (ref³⁴).

Persistent gut humoral memory; long-lived memory B cells and plasma cells

Persistent humoral memory to gut pathogens and other gut antigens can be obtained by long-lived memory B cells replenishing dying plasma cells or by plasma cells which themselves are long-lived. The possibility of long-lived gut plasma cells was not considered until recently as an early study of mice established the notion that gut plasma cells are short lived with an average turnover of a few days with maximal life span of a few weeks. Staining of rotavirus specific plasma cells in healthy individuals with no obvious recent history of rotavirus infection, however gave an indication that there could be longevity among gut plasma cells.^{17, 20} The fact that some but not all IgA and IgM plasma cells survive in biopsy cultures after 4 weeks without signs of proliferation supports the concept that there could be long-lived plasma cells in the gut, but also cells with shorter life spans.¹⁸ Gut plasma cells lack CD95 and have higher mRNA expression levels of *Bcl-2*, *Blimp-1*, *IRF-4* and *XBP-1* than intestinal memory B cells further indicating that the gut harbors long-lived plasma cells.²⁰ Future studies are needed to establish whether there are distinct populations of plasma cells with regards to life spans and also how long plasma cells survive in the gut.

The importance of memory B cells for gut humoral immunity has been extensively studied in the mouse and recently some relevant human studies have also appeared. High throughput sequence analysis of immunoglobulin heavy chain genes of cells in colon biopsies from healthy human subjects obtained before the start of, four days after and 46 days after cessation of antibiotic treatment revealed the same repertoire of expanded and non-expanded clusters of clonally related IgA sequences in all samples. Backed up by parallel studies in mice where memory B cells were seen to circulate between inductive compartments with clonal sharing of plasma cells in gut and mammary glands, this longitudinal persistence of clonally related B cells in the IgA repertoire despite major changes in the microbiota was taken to support the notion of a continuous diversification of memory B cells and this was considered

a central element in the shaping of the gut humoral response.⁷⁷ IgA plasma cells of duodenal biopsies of celiac disease patients not reactive to transglutaminase (i.e. covering most of the repertoire) on average have 10% related sequences among IgA CD27+ peripheral blood B cells also pointing to clonal relatedness between memory B cells and gut plasma cells.⁸⁶ Based on available data it hence seems likely that long-lived B cell responses initiated in GCs do exist in humans, but the relative contribution of long-lived circulating memory B cells and long-lived plasma cells in the lamina propria to maintain a persistent gut humoral response awaits clarification.

Existence of plasma cell survival niches in the intestine

It is well established that in the bone marrow and in inflamed tissues there are plasma cells with very long life spans. The maintenance of long-lived plasma cells in the bone marrow is dependent on plasma cells settling into survival niches made up of stromal cells and cytokines (Reviewed in ^{87,88}). Stromal cell expression of CD44 and the chemokine CXCL12 (binding CXCR4 on plasma cells) and the cytokines IL-6, TNF, APRIL and BAFF (the two latter binding BCMA on plasma cells) are key components of such niches. There is competition for a finite number survival niches and which cells make it into a niche is determined by stochastic processes and plasma cell intrinsic factors – their competence to compete. Having entered the niche, the plasma cells express high levels of the transcription factors *Blimp-1*, *XBp-1*, and *IRF-4*. Mouse studies have suggested that eosinophils producing APRIL are important for plasma cell survival in the bone marrow and for maintenance of IgA plasma cells in the gut, and presence of plasma cells adjacent to eosinophils in human intestine might suggest a similar role for eosinophils in man.⁸⁹ (Figure 4) Interestingly, culturing of gut biopsies without tissue disruption gave the highest antibody production and plasma cell survival indicating a role of the tissue environment also for gut plasma cell longevity.¹⁸ In these gut biopsy cultures, IL-6 and APRIL were detected and blocking of the activity of endogenous APRIL and IL-6 with

BCMA-Fc anti-human IL-6 antibody negatively affected antibody secretion suggesting a role for these cytokines in gut plasma cell survival. By immunohistochemistry and quantitative real-time PCR, APRIL expression in the lamina propria was found in macrophages, dendritic cells and neutrophils.²³ Scarce or no expression of APRIL was seen in the areas of epithelium adjacent to gut lumen, but APRIL was intensely expressed by crypt epithelial cells (Figure 6). Likewise, mRNA for the APRIL receptors TACI and BCMA was detected in microdissected lamina propria tissue. By quantitative real-time PCR of lamina propria tissue devoid of lymphoid aggregates, expression of APRIL and BAFF was found in both fetal and adult samples and there was distinct expression of BCMA in adult samples and little expression of BAFF-R and TACI in both types of samples.²⁴ By flow cytometry analysis of celiac disease biopsies, BCMA was expressed on most plasma cells, whereas BAFF-R and TACI were expressed only by a subgroup of cells and at variable levels. As for IL-6, this cytokine is produced by human small intestinal epithelial and smooth muscle cell lines in vitro and such cells could be sources for IL-6 in the intact intestinal tissue.⁹⁰ Immunohistochemical analysis suggests that CXCL12 is constitutively expressed in the intestine by enterocytes, but not by stromal cells, and it is up-regulated upon inflammation.⁹¹ Taken together it thus appears that many components of plasma cells survival niches as seen in the bone marrow exist in the gut (Figure 4).

Lessons from pathology and disease

Insight into the gut humoral immune system does not only come from studies of healthy subjects. Observations from patients with celiac disease in particular have given useful insights.

Celiac disease is characterized by highly disease specific IgA and IgG antibodies to the autoantigen transglutaminase 2 (ref ⁹²) and deamidated gluten (gliadin) peptides.⁹³ Anti-

transglutaminase antibodies and probably also anti-deamidated gluten antibodies are only produced in individuals who express HLA-DQ2 and /or HLA-DQ8 upon gluten consumption.⁹⁴ The levels of these antibodies in serum normalize within months after start-up of the gluten free diet.⁹⁵ The lesion localized in the proximal small intestine is characterized by blunting of intestinal villi and tissue leukocyte infiltration with a particularly striking increase in density of plasma cells.⁹⁶ The strong disease association with certain HLA-DQ allotypes and disease specific antibodies demonstrating the same genetic restriction points to crosstalk between T cells and B cells being involved in the pathogenesis.⁹⁷ Plasma cells specific for TG2 and deamidated gluten peptides can be isolated via staining with labelled antigen as they express surface immunoglobulin. On average 10% of the plasma cells in the active celiac disease lesion are transglutaminase 2 specific with less cells being specific for deamidated gluten.^{78, 80, 86} The number of transglutaminase 2 specific plasma cells drops quickly after start-up of the gluten free diet, but interestingly they do not disappear completely.⁹⁸ There is high degree of clonal sharing of transglutaminase 2 specific plasma cells between distinct duodenal biopsies, up to 70% as assessed by high throughput sequencing of immunoglobulin VH genes, giving support to the notion of wide seeding of plasma cells in the small intestine. Moreover, there is poor correlation of density of IgA transglutaminase 2 specific plasma cells in the lesion and serum IgA anti-transglutaminase 2 antibody levels, suggesting that serum antibodies have a main source outside of the gut.⁹⁸ Both the transglutaminase 2 and the gluten specific plasma cells have fewer mutations than other plasma cells in the lamina propria. The reason for this is unknown, but may relate to these being extrafollicular responses or GC reactions of short duration. Access to GALT structures of celiac disease patients would help to dissect this, but ethical aspects represent clear restrictions on further research. The common low mutation rate of the responses to the two antigens could relate to common T-cell help; gluten reactive and HLA-DQ2/DQ8 restricted T cells could provide help to both transglutaminase 2 specific and deamidated gluten specific B cells via transglutaminase-gluten peptide complexes.⁹⁹

Likewise B cells specific for the two antigens could serve as antigen presenting cells for gluten reactive T cells. Thus the abundance of transglutaminase 2 and deamidated gluten specific plasma cells and the antibodies being good diagnostic markers for disease could be a reflection of B cells playing an important role as antigen presenting cells amplifying the anti-gluten T-cell response rather than the antibodies themselves having a pathogenic role.⁹⁷

Outlook

Despite substantial insight into the generation, dissemination and regulation of the plasma cell population of the human gut there is still much to be learned. Available evidence suggests that the repertoire of human gut IgA plasma cells is formed in response to specific recognition of antigens, but under unique molecular control. With the arrival of high throughput technologies that allow for analysis of B-cell receptor genes and transcriptional programs at the single cell level even of cells specific for given antigens, it is to be expected that the near future will give interesting new knowledge of the human intestinal B-cell system. This should help us to make more efficient mucosal vaccines and to obtain a better understanding of gut immune disorders.

Figure Legends

Figure 1. Immune responses in the intestine are initiated in GALT and in gut regional lymph nodes. Whereas food and ingested antigens are present in the small bowel, microbial antigens represent a major immune challenge in the colon. Antigens at both gut locations are sampled by FAE. Dendritic cells in the subepithelial area are key regulators of the fate of responding B cells. They express the enzyme retinaldehyde dehydrogenase that catalyzes the production of retinoic acid that subsequently imprints homing via the induction of $\alpha 4\beta 7$ and CCR9. They also secrete IGIP and APRIL that together with stromally derived TGF β support IgA class switch recombination. Following clonal expansion, hypermutation and selection in the germinal centers of Peyer's patches or regional lymph nodes, plasmablasts enter the blood, via the lymphatic system. Plasmablasts subsequently home back to the lamina propria where they differentiate into plasma cells. The chemokines CCL25 and CCL28 may contribute to the differential homing of plasmablasts primed in the small intestine or colon, though how and where the lymphocyte receptor of CCL28, CCR10 is induced is not known.

Figure 2. Paraffin sections of GALT in human colon stained for A. CD20 (brown) B. IgD (brown) and C. CD3 (pink) and CD8 (brown). Nuclei are counterstained blue. The GC is surrounded by a dashed circle. Highly IgD expressing naïve B cells in B surround the GC, forming a relatively sparse mantle zone. The more extensive marginal zone B cell population extends between the mantle zone and the FAE. T cells in GALT are mostly CD4⁺ cells. T follicular helper cells defined by their location in the GC are rarely CD8⁺ and are located predominantly in the light zone (LZ) of the GC where they are involved in repertoire selection. T cells are rarely present in the dark zone (DZ).

Figure 3. Paraffin section of human ileum stained with CD20 (brown). Nuclei are counterstained in blue. CD20 expressing B cells are abundant in GALT. The lamina propria

adjacent to GALT may contain B cells that tend to be large cells resembling plasmablasts (identified with a box in A and magnified in B). Distant lamina propria however contains few, if any, CD20+ B cells (box C).

Figure 4. Illustration of factors that support plasma cells that can bind antigen through their B cell receptor in the lamina propria niche. Stromal cell derived IL-6 may bind to plasma cell IL-6 receptor and APRIL produced by epithelial cells, macrophages or eosinophils may bind to BCMA on plasma cells to support plasma cell survival. CXCL12 may promote plasma cell retention and survival through binding CXCR4. Plasma cell frequencies in intestine may also be regulated by T cells through the presentation of cognate antigen to local T cell populations.

Figure 5. A. Paraffin section of GALT from ileum stained for APRIL (brown) and AID (pink). Nuclei are counterstained with haematoxylin (blue). The GC is identified with a dashed line. B. AID staining is confined to the GC with the exception of occasional large interfollicular B cells that have cytoplasmic AID. APRIL is produced adjacent to B cells expressing AID in the GC. C. APRIL is abundant in cells with dendritic and macrophage morphology beneath the FAE. D. An area where the crypt epithelium expressing APRIL abuts the lymphoid tissue is indicated with a dotted line. Areas magnified in B, C and D are identified with labeled boxes in A.. E. The receptors for soluble APRIL and BAFF are illustrated schematically for reference.

Figure 6. Paraffin sections of human ileum (A and B) and colon (C, D and E) stained for IgA (A) and APRIL (B, C, D and E). IgA plasma cells (arrow heads) secrete IgA that is transported into the gut lumen following binding to the pIgR on the basolateral surface of epithelial cells. IgA in transit through epithelial cells is indicated with an arrow. In small intestine APRIL, like the pIgR, is expressed mostly in by epithelial cells in the bases of the crypts. In contrast, in colon, APRIL is expressed by subepithelial macrophages (D. Magnified area identified as a box C)

and epithelial cells (E). As in the ileum, epithelial APRIL expressed mostly by crypt cells in colon.

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